Development of a Next Generation Sequencing Panel Targeting Cannabinoid Synthase Genes to Distinguish Between Marijuana and Hemp

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Cannabis sativa, which can be grown as marijuana or hemp, has been one of the most contentious plants in the US. Law enforcement is now tasked with identifying the two types of crops as a result of the 2018 Agricultural Improvement Act, which legalized hemp. Genetic markers are desirable for determining crop type in sample types that are inconducive to chemical analysis, such as trace residues, small leaf fragments, immature crops, aged samples, seeds, and root material. Cannabinoid synthase genes are considered the key enzymes that determine the chemical composition or chemotype of a particular cultivar. Several researchers have suggested the use of cannabinoid synthase genes, particularly tetrahydrocannabinolic acid synthase (THCAS) and cannabidiolic acid synthase (CBDAS), to forecast the chemotype of *Cannabis sativa*.

A wider range of cannabis types must be taken into account when evaluating cannabis-related genetic markers because existing techniques for crop type identification based on previous theories may lack specificity and precision. The cannabis genome has been revealed to include several copies of cannabinoid synthase genes. The majority of homologs are pseudogenes with nucleotide identities between 91 and 95 percent to the functioning synthase genes. Therefore, the risk of amplification of pseudogenes cannot be overlooked in all synthase-gene targeting methods for the genetic testing of cannabis. This study evaluated not only THCAS and CBDAS but also synthase-like genes (pseudogenes), as well as another commonly seen synthase gene, cannabichromene acid synthase (CBCAS). A custom next generation sequencing (NGS) panel was developed that allowed us to target the three cannabinoid synthase genes: THCAS, CBDAS, and CBCAS, as well as the pseudo synthase genes: THCAS-Like, CBDAS-Like, and CBCAS-Like, in a broad variety of *C. sativa* samples. Samples including reference *C. sativa* samples from the National Institute on Drug Abuse (NIDA) and National Institute of Standards and Technology (NIST), CBD, CBG, delta-8-THC, delta-10-THC, THCO, and CBDV hemp samples, hemp seed samples, and seized marijuana extracts from the Drug Enforcement Agency (DEA). This presentation will be demonstrating the development and analysis process using the NGS panel to discover novel polymorphisms and comprehensively evaluate the relationship between the cannabis chemotype and the synthase gene copies. The study suggested a novel flow chart for genetic distinction between hemp and marijuana. The knowledge gained from this study will also be incorporated to develop a fast-genotyping assay that could potentially assist law enforcement agencies in distinguish legal hemp and illicit marijuana.

Keywords
***Cannabis sativa*, Cannabinoid synthase gene, Next generation sequencing**